Amendments to the claims

Please amend the claims as follows:

Please cancel claim 67, without prejudice or disclaimer.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claims 1 to 30 (canceled)

Claim 31 (previously presented): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

(a) providing an aldehyde or a ketone;

(b) providing a cyanide-containing compound;

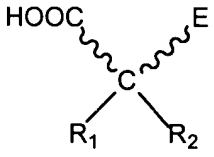
(c) providing an ammonia-containing compound or a compound comprising an ammonium salt or an amine;

(d) providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or an enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4;

(e) contacting the aldehyde or ketone of step (a) with a cyanide-containing compound of step (b) and an ammonia-containing compound or a compound comprising an ammonium salt or an amine of step (c) such that an amino nitrile or a cyanohydrin intermediate is produced; and

(f) contacting the amino nitrile or cyanohydrin intermediate of step (e) with the composition of step (d) such that the nitrilase stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid,

wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy,

alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or a lower alkyl.

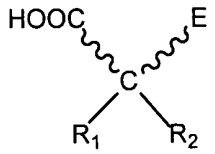
Claim 32 (previously presented): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

(a) providing a composition comprising an amino nitrile or a cyanohydrin;

(b) providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4; and

(c) contacting the amino nitrile or cyanohydrin of step (a) with the composition of step (b) such that the nitrilase stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid,

wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl.

Claims 33 to 35 (canceled)

Claim 36 (currently amended): A method for stereoselectively producing an alpha-amino acid, the method comprising

(a) providing an aldehyde or a ketone;

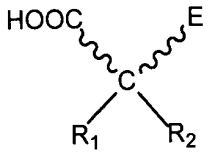
(b) providing a cyanide-containing compound and ammonia;

(c) providing a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2, or SEQ ID NO:4;

(d) contacting the aldehyde or ketone of step (a) with the cyanide-containing compound and ammonia of step (b) such that an amino nitrile is produced; and

(e) contacting the amino nitrile of step (d) with the nitrilase of step (c) such that the nitrilase stereoselectively hydrolyzes the amino nitrile to produce an alpha-substituted carboxylic ~~amino~~ acid,

wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl.

Claim 37 (previously presented): The method of claim 31, 32 or 36, wherein the reaction takes place in a single reaction vessel.

Claims 38 to 43 (canceled)

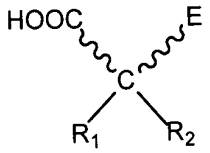
Claim 44 (currently amended): A method for producing an alpha-substituted carboxylic acid, said method comprising

contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound, an ammonium salt or an amine, and

hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with a nitrilase, wherein the nitrilase hydrolyzes the reaction components to produce an alpha-substituted carboxylic acid and

wherein the nitrilase has (i) an amino acid sequence having at least 90% [[80%]] sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 90% [[80%]] sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid encodes a nitrilase [[an]] enzyme ~~that retains the same enzymatic activity as the enzyme encoded by the nucleic acid sequence from which it varies~~,

wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl.

Claims 45 to 48 (canceled)

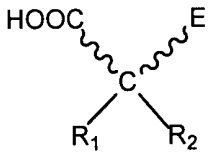
Claim 49 (previously presented): A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or a enzymatically active fragment thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4; and

contacting reaction components with the composition such that the nitrilase stereoselectively hydrolyzes the reaction components to produce an alpha-substituted carboxylic acid,

wherein the reaction components are an aldehyde or ketone, a cyanide-containing compound, and an ammonia-containing compound, ammonia salt, or amine,

wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl.

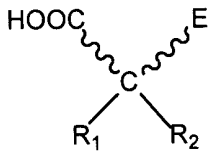
Claim 50 (currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising hydrolyzing stereoselectively the reaction components with a nitrilase,

wherein the reaction components are an aldehyde or ketone, a cyanide-containing compound, and an ammonia-containing compound, ammonia salt, or amine,

wherein the nitrilase has (i) an amino acid sequence having at least 90% **[[80%]]** sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 90% **[[80%]]** sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3,

wherein the nucleic acid encodes an enzyme that retains the same enzymatic activity as the enzyme encoded by the nucleic acid sequence from which it varies,

wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl.

Claim 51 (canceled)

Claim 52 (previously presented): The method of claim 31, 32, or 36, wherein said α-substituted carboxylic acid is an α-amino acid.

Claim 53 (previously presented): The method of claim 31, 32, or 36, wherein at least one of R_1 and R_2 is substituted or unsubstituted aryl.

Claim 54 (previously presented): The method of claim 52, wherein said α-amino acid is D-phenylalanine, D-phenylglycine, or L-methylphenylglycine.

Claim 55 (previously presented): The method of claim 52, wherein said α-amino acid bears a substituted or unsubstituted alkyl side chain.

Claim 56 (previously presented): The method of claim 52, wherein said α-amino acid is L-tert-leucine, D-alanine, or D-hydroxynorleucine.

Claim 57 (previously presented): The method of claim 31, 32, or 36, wherein said α-substituted carboxylic acid is an α-hydroxy acid.

Claim 58 (previously presented): The method of claim 57, wherein said α-hydroxy acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.

Claim 59 (previously presented): The method of claim 31 or 36, wherein said cyanide-containing compound comprises a metal cyanide or a gaseous cyanide.

Claim 60 (previously presented): The method of claim 59, wherein said cyanide-containing compound comprises an alkali cyanide.

Claim 61 (previously presented): The method of claim 59, wherein said cyanide-containing compound is sodium cyanide.

Claim 62 (previously presented): The method of claim 31, 32, or 36, wherein said ammonia salt has the formula $NH_2(R)_2^+X$, wherein each R is independently -H or lower alkyl, and X is a counter ion.

Claim 63 (previously presented): The method of claim 62, wherein X is a halide.

Claim 64 (previously presented): The method of claim 63, wherein said halide is Cl^-.

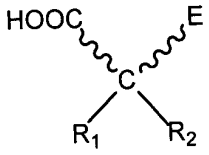
Claim 65 (previously presented): The method of claim 64, wherein said ammonia salt is $NH_4^+Cl^-$.

Claim 66 (currently amended): A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising

(a) providing a polypeptide having nitrilase activity, wherein the nitrilase is encoded by a nucleic acid that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3, and the stringent hybridization conditions comprise hybridization in a solution comprising 0.1 5M NaCl, 10% formamide, for 15 minutes at 72°C, and a wash step comprising a wash in a buffer comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na_2EDTA, 0.5% SDS, at room temperature for 30 minutes;

(b) contacting a composition comprising an aldehyde or ketone moiety with a cyanide-containing compound and an ammonia- containing compound, an ammonium salt or an amine, thereby producing an amino nitrile or cyanohydrin intermediate; and

(c) hydrolyzing stereoselectively the resulting amino nitrile or cyanohydrin intermediate with the polypeptide having nitrilase activity to produce an alpha-substituted carboxylic acid, wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl~~, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen~~.

Claim 67 (canceled)

Claim 68 (currently amended): The method of claim 44 [[67]], wherein the nitrilase has (i) an amino acid sequence having at least 95% [[90%]] sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 90% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

Claim 69 (currently amended): The method of claim 50 [[68]], wherein the nitrilase has (i) an amino acid sequence having at least 95% sequence identity to an amino acid sequence

consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 95% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

Claim 70 (previously presented): The method of claim 69, wherein the nitrilase has (i) an amino acid sequence having at least 97% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 97% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

Claim 71 (currently amended): The method of claim 68 ~~44 or claim 50~~, wherein the nitrilase has an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, and having at least one conservative amino acid substitution from the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, and has at least 97% **[[80%]]** sequence identity to the sequence of SEQ ID NO:2 or SEQ ID NO:4.

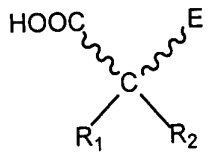
Claim 72 (currently amended): A method for producing an alpha-substituted carboxylic acid, said method comprising

(a) providing a composition comprising an aldehyde or ketone, and a cyanide-containing compound and an ammonia- containing compound, an ammonium salt or an amine;

(b) providing a polypeptide having nitrilase activity, wherein the polypeptide has an amino acid sequence comprising (i) an amino acid sequence having at least 90% **[[85%]]** sequence identity to a sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence encoded by a nucleic acid having at least 90% **[[85%]]** sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3;

(c) contacting the composition with the cyanide-containing compound and the ammonia-containing compound, ammonium salt or amine under conditions wherein an amino nitrile or cyanohydrin intermediate is produced; and

(d) **[[(c)]]** hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with the polypeptide having nitrilase activity, thereby hydrolyzing the reaction components to produce an alpha-substituted carboxylic acid, wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each R_x is each independently -H or lower alkyl~~, -OH, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl or heterocyclic~~.

Claim 73 (currently amended): The method of claim 72, wherein the polypeptide having nitrilase activity has (i) an amino acid sequence having at least 95% **[[90%]]** sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 95% **[[90%]]** sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

Claim 74 (currently amended): The method of claim 73, wherein the polypeptide having nitrilase activity has (i) an amino acid sequence having at least 97% **[[95%]]** sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) is encoded by a nucleic acid having at least 97% **[[95%]]** sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

Claim 75 (previously presented): The method of claim 72, wherein the α-substituted carboxylic acid is D-phenylalanine, D-phenylglycine, L-methylphenylglycine, L-tert-leucine, D-alanine, D-hydroxynorleucine, R-pantolactone, 2-chloromandelic acid, (S)-mandelic acid, (R)-mandelic acid or (S)-cyclohexylmandelic acid.

Claim 76 (previously presented): The method of claim 72, wherein the polypeptide having nitrilase activity hydrolyzes the reaction components to stereoselectively produce an alpha-substituted carboxylic acid.

Claim 77 (previously presented): The method of claim 44, wherein the nitrilase stereoselectively hydrolyzes the resulting amino nitrile or cyanohydrin intermediate to produce an enantiomerically pure alpha-substituted carboxylic acid.

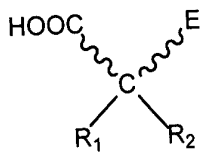
Claim 78 (currently amended): A method for producing an alpha-substituted carboxylic acid, said method comprising

(a) providing a polypeptide having nitrilase activity, wherein the polypeptide (i) is encoded by a nucleic acid that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3, and the stringent hybridization conditions comprise hybridization in a solution comprising 0.1 5M NaCl, 10% formamide, for 15 minutes at 72°C, and a wash step comprising a wash in a buffer comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na_2EDTA, 0.5% SDS, at room temperature for 30 minutes, or, (ii) has an amino acid sequence having at least 85% sequence identity to a sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4;

(b) providing a composition comprising an aldehyde or ketone moiety;

(c) contacting the composition with a cyanide-containing compound and an ammonia-containing compound, an ammonium salt or an amine, thereby producing an amino nitrile or cyanohydrin intermediate; and

(d) hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with the polypeptide having nitrilase activity, thereby hydrolyzing the reaction components to produce an alpha-substituted carboxylic acid, wherein said α-substituted carboxylic acid has the following structure:



wherein:

R_1 and R_2 are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety, and

E is $-N(R_x)_2$ or -OH, wherein each E and R_x is each independently -H or lower alkyl, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen.

Claim 79 (previously presented): The method of claim 78, wherein the polypeptide having nitrilase activity hydrolyzes the reaction components to stereoselectively produce an alpha-substituted carboxylic acid.

Claim 80 (previously presented): The method of claim 79, wherein the polypeptide having nitrilase activity stereoselectively hydrolyzes the resulting amino nitrile or cyanohydrin intermediate to produce an enantiomerically pure alpha-substituted carboxylic acid.